

# Macroscopic orientation effects in broadband NMR-spectra of model membranes at high magnetic field strength

## A method preventing such effects

T. Brumm, A. Möps,\* C. Dolainsky, S. Brückner, and T. M. Bayerl

Technische Universität München, Physik Department E22, D-W 8046 Garching; and \*Universität Leipzig, Sektion Physik, D-O 7010 Leipzig, Linnéstr. 5, Germany

**ABSTRACT** The partial orientation of multilamellar vesicles (MLV) in high magnetic fields has been studied and a method to prevent such effects is herewith proposed. The orientation effect was measured with  $^2\text{H}$ -,  $^{31}\text{P}$ -NMR and electron microscopy on MLVs of dipalmitoyl phosphatidylcholine with 30 mol% cholesterol. We present the first freeze—etch electron microscopy data obtained from MLV samples that were frozen directly in the NMR magnet at a field strength of 9.4 Tesla. These experiments clearly show that the MLVs adopt an ellipsoidal (but not a cylindrical) shape in the magnetic field. Best fit  $^{31}\text{P}$ -NMR lineshape calculations assuming an ellipsoidal distribution of molecular director axes to the experimentally obtained spectra provide a quantitative measure of the average semiaxis ratio of the ellipsoidal MLVs and its change with temperature. The application of so-called spherical supported vesicles (SSV) is found to prevent any partial orientation effects so that undistorted NMR powder pattern of the bilayer can be measured independently of magnetic field strength and temperature.

The usefulness of SSVs is further demonstrated by a direct comparison of spectral data such as  $^{31}\text{P}$ - and  $^2\text{H}$ -NMR lineshapes and relaxation times as well as  $^2\text{H}$ -NMR dePaked spectra obtained for both model systems. These experiments show that spectral data obtained from partially oriented MLVs are not unambiguous to interpret, in particular, if an external parameter such as temperature is varied.

## INTRODUCTION

NMR studies of phospholipid multilamellar vesicles in which the tensor interactions (e.g., chemical shift, dipolar, and/or quadrupolar interactions) are not averaged to zero are well established methods to provide information about the adopted structure and about the molecular dynamics of such model membrane systems (1–4).

However, phospholipids are well known to become orientated in an applied static magnetic field due to the anisotropy of diamagnetic susceptibility  $\Delta\chi_a$ , which arises mainly from their fatty acyl chains (5, 6). For phospholipids,  $\Delta\chi_a$  is generally negative, its exact value depends on the molecular structure. Since  $\Delta\chi_a$  is an additive property, its total value can be large in regular arrays such as bilayers. As a consequence, ellipsoidal and/or cylindrical vesicles are formed in the homogeneous static magnetic field  $H_0$  of an NMR spectrometer with their long axes aligned parallel to  $H_0$  (7–9). In the case of cylindrical vesicles, thermal motions cause fluctuations of the cylinder long axes around  $H_0$ , where each state is populated according to a Boltzmann distribution  $w(\theta, \phi)$ :

$$w(\theta, \phi) \sin \theta \, d\theta \, d\phi \sim \exp(-a \sin^2 \theta) \sin \theta \, d\theta \, d\phi. \quad (1)$$

The mean degree of orientation  $a$  is given by

$$a = c_0 \Delta\chi_a H_0^2 / (kT), \quad (2)$$

and the angle  $\theta$  is the polar angle of the long axis of the cylinder with respect to  $H_0$ . The constant  $c_0$  accounts for the geometry of the vesicle (7):

$$c_0 = lrb\pi/2, \quad (3)$$

where  $l$  is the length,  $r$  the radius of the cylinder, and  $b$  the bilayer thickness. On the other hand, the rapid technical progress in the construction of cryomagnets has led to the widespread availability of NMR spectrometers with magnetic field strengths up to 15T. For solid state NMR experiments on model membranes, field strengths of 9.4T (corresponding to a proton frequency of 400 MHz) are often used. From Eq. 2 it is obvious that the degree of alignment of cylindrical phospholipid vesicles scales with the square of the magnetic field strength. Thus, partial orientation effects of phospholipid vesicles, which are negligible at lower field strengths such as 4.6T (200 MHz), may become a dominant feature of spectra obtained at higher fields. As a consequence, some spectral data obtained from such partially oriented spectra may differ from those of true spherical vesicles. The most noteworthy examples of spectral data, which may change due to partial orienta-

Address correspondence to Dr. Thomas M. Bayerl, Technische Universität München, Physik Department E22, D-W 8046 Garching, Germany

tion, are NMR lineshapes, moments, and relaxation times. Moreover, the calculation of oriented spectra from the measured powder pattern using iterative methods which rely on assumptions about the spherical geometry of the sample, such as the dePakeing of  $^2\text{H}$ -NMR spectra, may become an obstacle.

There are two aims of this paper. Firstly, we present evidence that multilamellar vesicles of DPPC/cholesterol adopt a predominantly ellipsoidal shape at the magnetic field strength used in this study (9.4T, 400 MHz proton NMR frequency) due to magnetic field orientation. Secondly, we propose a method that completely eliminates these partial orientation problems so that the vesicles maintain their spherical shape. The latter is achieved by application of a recently developed model system, the so-called spherical supported vesicles (SSV) (10). SSVs are spherical silica beads with a narrow distribution of sizes. Each bead is surrounded by a single spherical bilayer separated from the surface by a thin water layer. In contrast to low hydrated multilayer samples oriented between glass plates, which can also be used to prevent macroscopic orientation by magnetic fields, the SSV samples can be applied to the same water concentration as the MLVs, i.e., as aqueous dispersions.

We demonstrate the advantages of SSV samples as compared with the commonly used multilamellar vesicles (MLV) concerning partial orientation effects. This is achieved by a direct comparison of spectral data such as  $^{31}\text{P}$ - and  $^2\text{H}$ -NMR lineshapes and relaxation times, as well as  $^2\text{H}$ -NMR dePaked spectra obtained for both model systems using a binary mixture of dipalmitoylphosphatidylcholine (DPPC) with 30 mol% cholesterol. MLVs consisting of this mixture exhibit a significant temperature-dependent partial field orientation effect. Lineshape calculations assuming an ellipsoidal shape of such MLVs were performed with various semiaxis ratios in order to obtain a quantitative measure of the degree of their macroscopic magnetic field orientation with temperature. In contrast, the SSVs are found to maintain their highly defined spherical shape under the same conditions so that true, undistorted powder pattern can be measured.

## EXPERIMENTAL

### Samples

1,2-perdeuterio-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC- $d_{62}$ ) was obtained from Avanti Polar Lipids Inc. (Alabaster, Alabama) and cholesterol was from Fluka (Buchs, Switzerland).

Multilamellar vesicle dispersions and highly oriented multibilayers were prepared according to procedures given in the paper of Reintj et al. (companion article). Spherical supported vesicles were prepared by condensation of small unilamellar vesicles on silica beads of  $640 \pm 45$  nm diameter according to previously published procedures (10). The

silica beads were kindly provided as a gift by Dr. Müller from the Degussa Research Laboratories (FRG). These beads are perfectly spherical as judged from electron microscopy.

## Methods

Deuterium NMR and phosphorus NMR experiments were performed at 61 and 161 MHz, respectively, using a Varian VXR-400 spectrometer (Palo Alto, CA) equipped with a high power probe. All  $^2\text{H}$ -NMR spectra were obtained using the quadrupolar echo technique with a pulse separation of 20  $\mu\text{s}$  and two  $90^\circ$ -pulses of 6  $\mu\text{s}$  duration. The repetition time for successive pulse sequences was 200 ms and 8K data points were collected with a dwell time of 1  $\mu\text{s}$ . All experiments were done on resonance with an 8 cyclops pulse cycling sequence (11) and no phase corrections were performed. For  $^{31}\text{P}$ -NMR experiments, the Hahn spin echo technique with a pulse separation of 20  $\mu\text{s}$ , a  $90^\circ$  pulse of 5.5  $\mu\text{s}$  duration, and a recycle delay of 3 s was used together with high power square wave proton decoupling. 1,000 scans were accumulated for the multilamellar vesicles and the oriented multibilayers. An exponential function corresponding to a linebroadening of 100 Hz was applied to the free induction decays before Fourier transform. For spherical supported vesicles, 5,000 scans were acquired. The temperature of the sample was controlled by a Varian temperature control unit and was constant within  $\pm 0.5^\circ\text{C}$ .

$^2\text{H}$ -NMR  $T_2^*$  measurements were performed by increasing the separation,  $\tau$ , between the  $\pi/2$  pulses in 10 steps up to 500  $\mu\text{s}$ . At each separation  $\tau$ , 1,000 scans were accumulated. Semilogarithmic plots of the normalized peak intensity of the echo versus  $2\tau$  were linear for both multilamellar vesicles and spherical supported vesicles. The slope of these plots gave  $(T_2^*)^{-1}$ .

The  $^{31}\text{P}$ -NMR lineshapes for spherical and ellipsoidal vesicle shapes were calculated by Fourier transformation of a simulated free induction decay (FID) according to procedures described in detail in the paper of Reintj et al. (companion paper). Gaussian line broadening and an orientation dependence of  $T_2$  of the form  $1/T_2 = A + B \sin^2 \theta \cdot \cos^2 \theta$  was applied to the simulation of the FID signal. The coefficients  $A$  and  $B$  were obtained by best fits of the experimental  $^{31}\text{P}$ -NMR  $T_2$  relaxation spectrum of the MLVs to this orientation dependence at the corresponding temperature:  $A^{-1} = 3$  ms,  $B^{-1} = 3$  ms at  $35^\circ\text{C}$ , and  $A^{-1} = 5$  ms,  $B^{-1} = 3$  ms at  $50^\circ\text{C}$ . The effective chemical shift anisotropy of  $\Delta\sigma_{\text{eff}} = 7.0$  kHz used in the simulations was obtained from the SSV  $^{31}\text{P}$ -NMR spectrum in Fig. 2 C.

The calculation of the oriented  $^2\text{H}$ -NMR spectra (bilayer normal parallel to  $H_0$ ) was achieved using an interactive dePakeing program written in the laboratory of Professor Myer Bloom by Dr. E. Sternin (University of British Columbia, Vancouver, Canada).

Freeze-etch electron microscopy replicas were obtained by rapidly freezing the sample in liquid propane down from a temperature of  $35$ – $40^\circ\text{C}$  right at the position inside the NMR cryomagnet where the NMR sample tube is usually placed. The sample was incubated in the magnet at  $35$ – $40^\circ\text{C}$  for 30 min before freezing in order to allow equilibration of the vesicle shapes in the magnetic field. We found that longer equilibration times did not increase the degree of partial field orientation. The home built freezing device consisted of a liquid nitrogen cooled reservoir of liquid propane thermally insulated by a 10-cm foam layer from a 2-cm thick thermostated aluminum disk (70 mm diameter) horizontally arranged on top of the reservoir. The freeze etch sample holder (gold planchettes from Balzers, FRG) containing a small droplet of the MLV sample was placed at the center of the disk that was kept at a temperature of  $35^\circ\text{C}$  by an external water bath. After incubation of the sample, a remote-controlled trap door mechanism inside the aluminum disk was activated, which caused the

sample to drop directly into the liquid propane reservoir below the disk. The obtained frozen samples were processed in a balzers freeze-etch device according to procedures described in detail elsewhere (12). The remote-controlled drop of sample into the liquid propane caused motion in the MLV dispersion such that the orientational order between the ellipses (alignment of the semimajor axes) established by the magnetic field was lost. Hence, the replicas showed ellipsoidal MLVs but with randomly oriented semimajor axes.

## RESULTS AND DISCUSSION

Earlier NMR papers which demonstrated the effect of the partial magnetic field orientation of phospholipid multilamellar vesicles on the NMR lineshape (8, 9) do not provide any evidence about the shape of the vesicles in the magnetic field of the spectrometer. Basically, one can expect that the vesicles undergo a transformation from a spherical shape into either a cylindrical or ellipsoidal one in the magnetic field. Scholz et al. (7) showed by optical microscopy that selected thin walled DMPC vesicles (uni- or bilamellar) exhibit a nearly cylindrical shape with the long axis aligned parallel to the field direction. However, MLVs in a strong magnetic field are likely to adopt an ellipsoidal shape because the higher bending stiffness of the highly curved inner shells of an MLV is expected to oppose the formation of cylinders.

A method which would permit an unambiguous decision about the shape MLVs adopt in a magnetic field is a fast freezing of the MLVs (e.g., in liquid propane) starting at a well defined temperature within the magnetic field and the examination of the obtained samples using freeze-etch electron microscopy. Therefore, we constructed a special device which enables the fast freezing of MLVs in the 9.4T wide bore superconducting magnet of our NMR spectrometer right at the place where the NMR sample tube is usually positioned. Representative freeze-etch replicas of MLV samples obtained using this device are given in Fig. 1 *A* and *B*. All MLVs of each sample exhibit an ellipsoidal shape, no cylindrical MLVs can be observed. A comparison of Fig. 1, *A* and *B*, shows that the ellipsoidal shape (but not the eccentricity) of the MLVs is independent of their size, as both MLVs differ in their diameter by nearly an order of magnitude. In fact, the MLV in Fig. 1 *B* is larger than the spacing of the electron microscopic grid such that only one-half of it can be reproduced. However, the eccentricity of the ellipses is found to be dependent on their size. The small MLVs shown in Fig. 1 *A* exhibit a semiaxis ratio  $r_{ex} = a/b$  ( $a$  is the semimajor axis and  $b$  the semiminor axis) of  $r_{ex} \approx 1.2$ , the large MLVs such as the one shown in Fig. 1 *B* give  $r_{ex} \approx 1.8$ –2.3. In contrast, a control sample frozen without the presence of a mag-

netic field exhibits nearly spherically shaped MLVs (Fig. 1 *C*). These data prove unambiguously, that at the magnetic field strength of 9.4 Tesla (corresponding to a  $^1\text{H}$ -NMR spectrometer frequency of 400 MHz) of the NMR experiments presented below, the field causes the MLVs to adopt a time averaged ellipsoidal, but not a cylindrical shape.

Fig. 2 shows  $^{31}\text{P}$ - and  $^2\text{H}$ -NMR spectra of MLVs of a binary mixture of DPPC- $d_{62}$ /cholesterol (30 mol%) at two temperatures. A closer inspection of the spectra in Fig. 2, *A* and *B* clearly shows severe deviations of their lineshape from that expected for a spherical distribution of the molecular director axes. The intensity of the edges (corresponding to a perpendicular orientation of the director axis to the external magnetic field) is more pronounced while the shoulder intensity (corresponding to a parallel orientation of the director axis to the external magnetic field) is drastically reduced. This situation is typically found in aqueous dispersions of phospholipids at high magnetic field strengths, where the average shape of the vesicles changes from a spherical to an ellipsoidal one due to macroscopic magnetic field orientation (8, 9).

Moreover, a comparison of the spectra in Fig. 2, *A* and *B*, provides evidence that the degree of the macroscopic magnetic field orientation of the DPPC- $d_{62}$ /cholesterol MLVs depends drastically on the temperature with the eccentricity of the ellipses increasing with temperature. In order to obtain a quantitative measure of the degree of macroscopic magnetic field orientation, we calculated  $^{31}\text{P}$ -NMR spectra assuming an ellipsoidal distribution of the director axes (semimajor axis parallel to  $H_0$ ) and fitted them to the experimental spectra by varying the semiaxis ratio  $r_{ex} = a/b$ . We found a good match with these calculated spectra (*dotted lines* in Fig. 2) with the experimental one. Thus, from the calculated spectra we can estimate a temperature increase of 10°C for an increase in eccentricity of the ellipsoidal MLVs by 25%. The reasons for the temperature dependence of the shape of DPPC/cholesterol MLVs in high magnetic fields are discussed in detail in the paper of Reinl et al. (companion paper).

In contrast to MLVs, SSVs, which consist of a single bilayer of DPPC/cholesterol (30 mol%) supported by a well defined silica sphere of 640 nm diameter, do not exhibit any magnetic field orientation effects. This is demonstrated by the  $^{31}\text{P}$ - and  $^2\text{H}$ -NMR spectra of such SSVs shown in Fig. 2 *C*. The calculated  $^{31}\text{P}$ -NMR spectrum assuming a spherical distribution of director axes (*dotted lines*) matches exactly the experimental spectrum. We measured the SSV spectra at various temperatures (up to 80°C), but no deviations from a lineshape

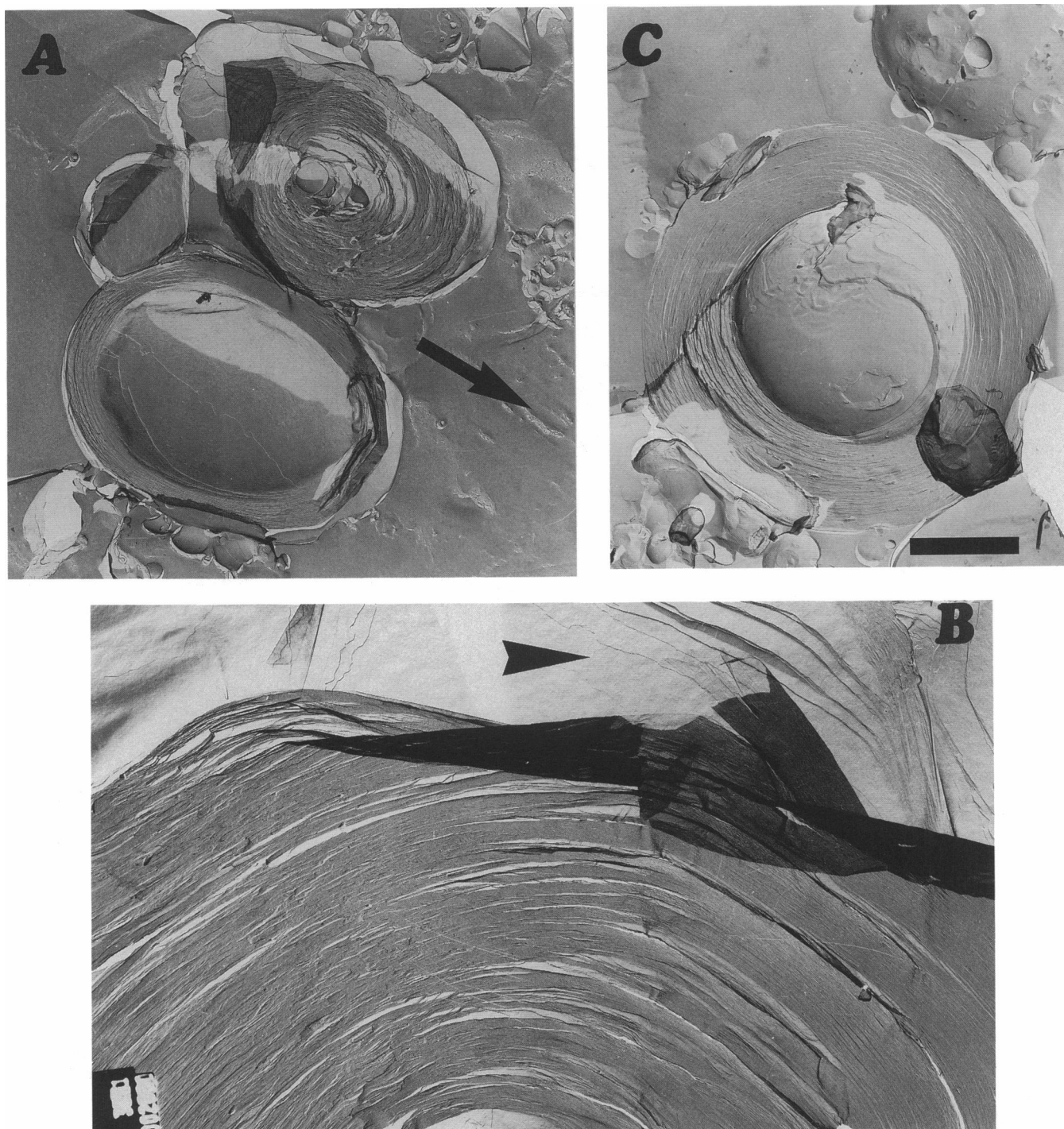


FIGURE 1. Freeze-etch replicas of multilamellar vesicles of DPPC- $d_{62}$ /cholesterol (30 mol%) fast frozen in liquid propane down from a temperature of  $37 \pm 2^\circ\text{C}$ . (A and B) Obtained at a magnetic field strength of 9.4 Tesla (arrows indicate the direction of the magnetic field and their length represents  $1\ \mu\text{m}$  [A] and  $2\ \mu\text{m}$  [B]). (C) Obtained with no magnetic field applied (bar represents  $1\ \mu\text{m}$ ).

characteristic for a spherical distribution of director axes was observed. Thus, the interaction energy between the single bilayer and the surface of the silica sphere is sufficiently strong to prevent any partial or total detach-

ment of the bilayer from the supporting sphere due to magnetic field orientation. Since the bilayer is separated from the silica surface by an ultrathin water layer of  $12\text{--}18\ \text{\AA}$  thickness (10, 13), this interaction force is

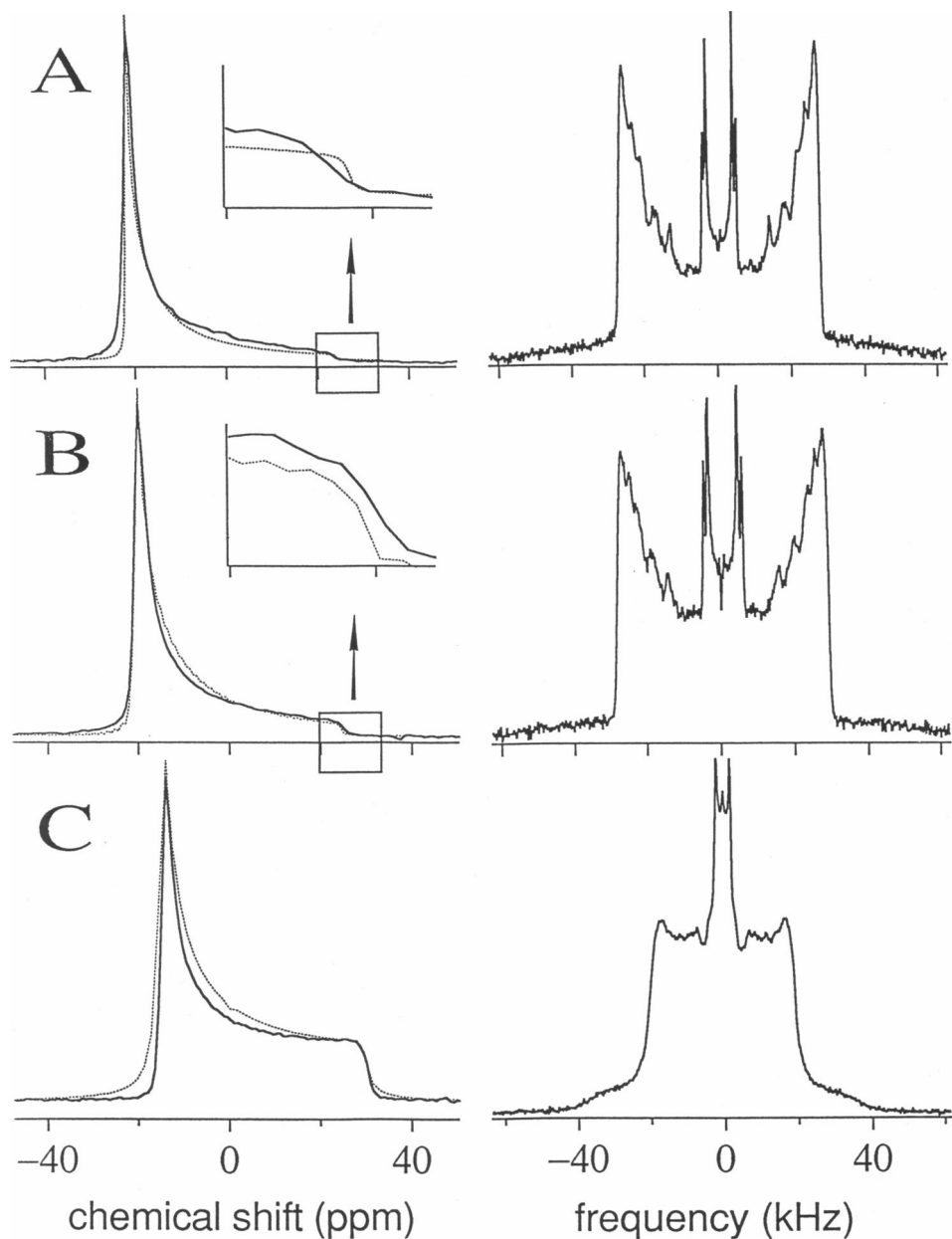


FIGURE 2.  $^{31}\text{P}$ - and  $^2\text{H}$ -NMR spectra of a binary mixture of DPPC- $\text{d}_{62}$ /cholesterol (30 mol%) obtained at 9.4T: multilamellar vesicles at (A)  $T = 50^\circ\text{C}$  and (B)  $T = 30^\circ\text{C}$ . For comparison, spherical supported vesicles at  $50^\circ\text{C}$  are shown in C. The dotted lines represent calculated spectra assuming an ellipsoidal distribution of director axes with a semiaxis ratio of  $r_{\text{ex}} = 2.4$  (A) and  $r_{\text{ex}} = 2$  (B) as well as assuming a spherical distribution of director axes (C).

presumably caused by a complicated superposition of attractive van der Waals forces, repulsive hydration forces, and attractive or repulsive Coulomb forces.

These results prove unambiguously that SSVs can be used to prevent (a) partial field orientation effects of phospholipid bilayers in high magnetic fields and (b) any changes of the lineshape with temperature due to a temperature dependence of the macroscopic magnetic

field orientation such as observed for MLVs (cf. Fig. 1, A, and B).

Hence, SSVs can be used as a standard for checking the reliability of spectral data and their changes due to the variation of external parameters such as temperature, ionic strength of the buffer medium, or the surface charge of the bilayer obtained from MLV samples as regards partial orientation effects. This advantage of the

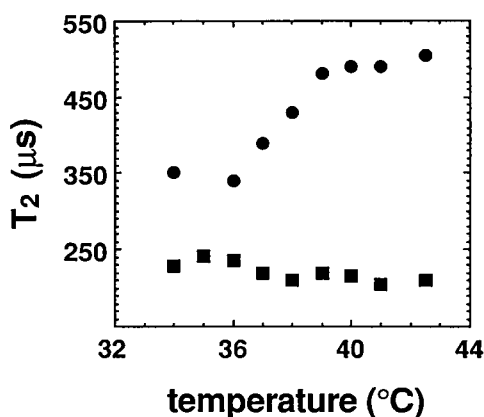


FIGURE 3. Temperature dependence of the  $^2\text{H}$ -NMR transverse relaxation time  $T_2^c$  of a bilayer of DPPC- $\text{d}_{62}$ /cholesterol (30 mol%) obtained for multilamellar vesicles (●) and for spherical supported vesicles (■).

SSV samples is clearly demonstrated by the examples given in the following, where we compared  $^2\text{H}$ -NMR spectral data obtained from SSVs and MLVs, respectively, of a binary mixture of DPPC- $\text{d}_{62}$ /cholesterol (30 mol%).

As  $^{31}\text{P}$ - and  $^2\text{H}$ -NMR spectra of phospholipid bilayer exhibit an orientationally anisotropic relaxation behavior, one can expect that changes of the partial orientation of the MLVs will change the orientationally averaged relaxation times determined from such spectra. The temperature dependence of the  $^2\text{H}$ -NMR transverse relaxation time,  $T_2^c$ , for both types of samples (MLV and SSV) is shown in Fig. 3. As discussed in an earlier paper (10), SSV samples exhibit  $T_2^c$  values significantly shorter than those obtained for MLVs. However, the temperature dependence of  $T_2^c$  for SSVs is more reliable because the shape of the vesicles is retained while the MLVs undergo a significant increase of their degree of magnetic field orientation in the same temperature range. The latter can explain the observed increase of  $T_2^c$  for MLVs: it has been shown that lateral diffusion of the phospholipids in MLVs provides a major  $T_2^c$  relaxation mechanism (14). The change of the ellipsoidal MLVs with increasing temperature towards a higher eccentricity with its long axis parallel to  $H_0$  diminishes the number of molecules in areas where the phospholipids can rapidly change orientation to  $H_0$  by lateral diffusion on the surface of the ellipse. Thus, the higher the eccentricity of the MLVs the lower the contribution of lateral diffusion to  $T_2^c$ , i.e., the average  $T_2^c$  must increase. Hence, the increase of the average  $T_2^c$  observed for MLVs (Fig. 3) may be an apparent one due

to a change of their magnetic field orientation and solely reflects the fact that  $T_2^c$  is orientation dependent (14, 15).

A further problem arising from the partial orientation of the MLVs is the calculation of an oriented spectrum from the powder pattern by a numerical dePakeing procedure (16). In  $^2\text{H}$ -NMR spectroscopy of bilayers consisting of phospholipids with perdeuterated acyl chains, this method can be used for the calculation of the order parameter profile along the acyl chains (17) and of

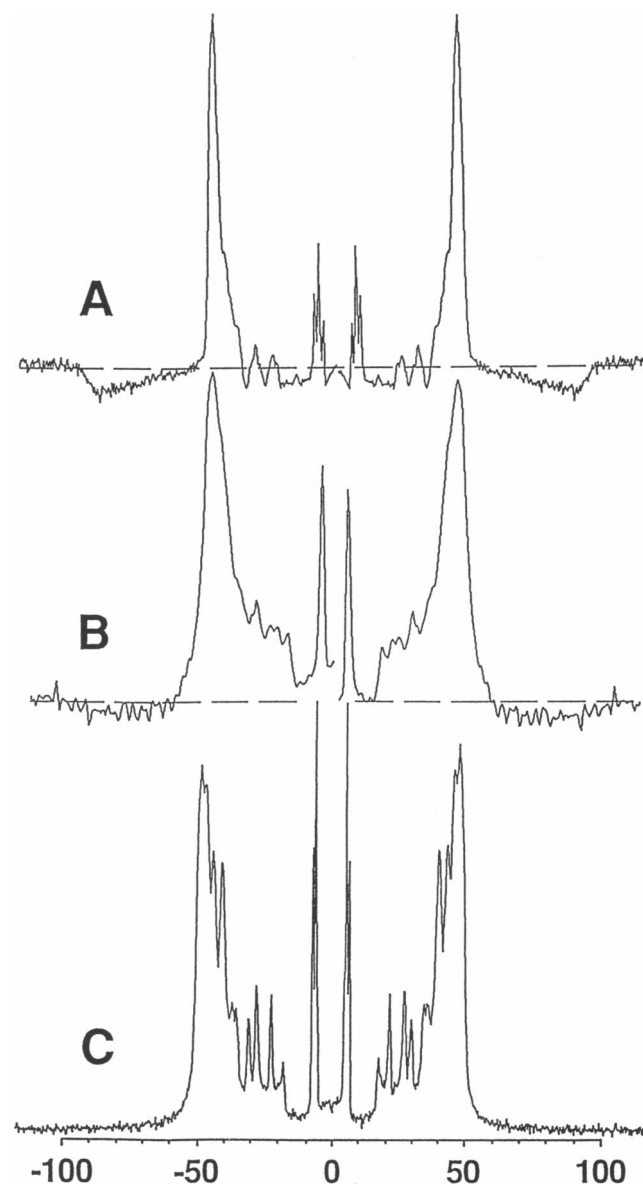


FIGURE 4. Oriented  $^2\text{H}$ -NMR spectra (membrane normal parallel to the external magnetic field) of a binary mixture of DPPC- $\text{d}_{62}$ /cholesterol (30 mol%) at 40°C: calculated by a dePakeing procedure from MLV (A) and SSV (B) powder pattern spectra and obtained experimentally from a highly oriented multibilayer sample (C).



the hydrophobic thickness of the membrane (2, 18). Since the numerical dePakeing algorithm relies on the assumption of a spherical distribution of director axes, base line problems in the calculated oriented spectra can be expected in the case of ellipsoidal MLVs. This is demonstrated in Fig. 4, where the dePaked spectra obtained from  $^2\text{H}$ -NMR powder pattern of MLVs and SSVs are compared with the experimentally obtained spectrum of a macroscopically highly oriented sample (multibilayers oriented between silicon wafers). The base line problems and the resulting lineshape obstruction of the oriented spectrum calculated from the MLV spectrum (Fig. 4 A) are obvious. The sag of the baseline of the oriented spectrum in Fig. 4 A can be observed at all positions along the fatty acyl chain, the bump at its wings corresponds to the reduced intensity of the shoulder region of the MLV spectrum. This debasement results in wrong intensities for each individual signal and makes some signals near the so-called plateau region (the signals arising from the positions C2–C11) unresolvable. In contrast, the dePaked SSV spectrum (Fig. 4 B) exhibits smaller baseline errors and agrees well (although less resolved) with the spectrum obtained from the oriented multilayer (Fig. 4 C). The lower resolution of the calculated SSV oriented spectrum (Fig. 4 B) as compared with that obtained from a highly oriented multilayer sample (Fig. 4 A) is at least partly caused by the significantly shorter transverse relaxation times of the SSVs (cf. Fig. 3).

## CONCLUSIONS

We have shown that multilamellar vesicles of DPPC/cholesterol undergo a significant temperature dependent partial orientation at a magnetic field strength of 9.4 Tesla, leading to an ellipsoidal shape of the vesicles. This partial orientation causes changes of the  $^2\text{H}$ -NMR spectral data (lineshape, transverse relaxation time) and leads to severe baseline problems of the dePaked spectra obtained from such distorted powder patterns. The application of SSVs completely prevents such partial orientation effects. The application of SSV samples in high field NMR spectroscopy of bilayer membranes seems particularly useful when studying lipid-protein interaction and related fields where the intercalation or adsorption of foreign molecules with phospholipid bilayers can change susceptibility anisotropy of the latter.

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## REFERENCES

1. Seelig, J. 1977. Deuterium magnetic resonance: theory and application to lipid membranes. *Quart. Rev. Biophys.* 10:353–418.
2. Seelig, J., and A. Seelig. 1980. Lipid conformation in model membranes and biological membranes. *Quart. Rev. Biophys.* 13:19–61.
3. Davis, J. H. 1983. The description of membrane lipid conformation, order and dynamics of  $^2\text{H}$ -NMR. *Biochim. Biophys. Acta.* 737:117–171.
4. Bloom, M. 1988. NMR studies of membranes and whole cells. *Phys. NMR Spec Biol. Med.* 121–157.
5. Boroske, E., and W. Helfrich. 1978. Magnetic anisotropy of egg lecithin membranes. *Biophys. J.* 24:863–868.
6. Kawamura, Y., I. Sakurai, A. Ikegami, and S. Iwanayagi. 1981. Magneto-orientation of phospholipids. *Mol. Cryst. Liq. Cryst.* 67:77–88.
7. Scholz, F., E. Boroske, and W. Helfrich. 1984. Magnetic anisotropy of lecithin membranes. *Biophys. J.* 45:589–592.
8. Seelig, J., F. Borle, and T. A. Cross. 1985. Magnetic ordering of phospholipid membranes. *Biochim. Biophys. Acta.* 814:195–198.
9. Speyer, J. B., P. K. Sripada, S. K. Das Gupta, G. G. Shipley, and R. G. Griffin. 1987. Magnetic orientation of sphingomyelin lecithin bilayers. *Biophys. J.* 687–691.
10. Bayerl, T. M., and M. Bloom. 1990. Physical properties of phospholipid bilayers adsorbed to micro glass beads. *Biophys. J.* 58:357–362.
11. Rance, M., and A. Byrd. 1983. Obtaining high-fidelity spin- $1/2$  powder spectra in anisotropic media: phase-cycled Hahn echo spectroscopy. *J. Magn. Reson.* 52:221–240.
12. Bayerl, T. M., T. Köchy, and S. Brückner. 1990. On the modulation of a high enthalpy pretransition in binary mixtures of DMPC and DMPG by polar headgroups interaction. *Biophys. J.* 57:675–680.
13. Johnson, S. J., T. M. Bayerl, D. C. McDermott, G. W. Adam, A. R. Rennie, R. K. Thomas, and E. Sackmann. 1991. Structure of an adsorbed dimyristoylphosphatidylcholine bilayer measured with reflection of neutrons. *Biophys. J.* 59:289–294.
14. Bloom, M., and E. Sternin. 1987. Transverse nuclear spin relaxation in phospholipid bilayer membranes. *Biochemistry.* 26:2101–2105.
15. Watnick, P. I., P. Dea, and S. Chan. 1990. Characterization of transverse relaxation rates in lipid bilayers. *Proc. Natl. Acad. Sci. USA.* 87:2082–2086.
16. Sternin, E., M. Bloom, and A. L. MacKay. 1983. De-pake-ing of NMR spectra. *J. Magn. Reson.* 55:274–282.
17. Lafleur, M., B. Fine, E. Sternin, P. R. Cullis, and M. Bloom. 1989. Smoothed orientational order profile of lipid bilayers by  $^2\text{H}$ -NMR. *Biophys. J.* 56:1037–1041.
18. Sankaram, M. B., and T. E. Thompson. 1990. Modulation of phospholipid acyl chain order by cholesterol. A solid-state  $^2\text{H}$ -NMR study. *Biochemistry.* 29:10676–10684.